

## STUDIES ON THE B VIRUS. I: THE IMMUNOLOGICAL IDENTITY OF A VIRUS ISOLATED FROM A HUMAN CASE OF ASCENDING MYELITIS ASSOCIATED WITH VISCERAL NECROSIS.

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AN unusual, fatal case of acute ascending myelitis in a laboratory worker following the bite of an apparently normal monkey was recently described (Sabin and Wright, 1934). Clinically, the striking features of the case were (a) the presence of vesicular, necrotic lesions at the sites of the bite on the two fingers, (b) regional lymphangitis and lymphadenitis, and (c) the development, 13 days after the bite of a transverse myelitis, which ascended and resulted in death within 4 days. Pathologically, in addition to a myelo-encephalitis, there was focal necrosis of the adrenals, spleen, and regional lymph-nodes. Ante-mortem and post-mortem studies failed to incriminate any bacterial infection as the cause of the disease. From the brain and cord as well as from the spleen of this case a filterable virus was isolated, which, when injected intracutaneously into rabbits, was capable of simulating the human disease both clinically and pathologically. Some of the properties of this virus have already been described (Sabin and Wright, 1934), and a more detailed study will be presented in a separate communication. For a better understanding of the immunologic studies to be described in this paper, it is perhaps necessary to mention briefly the main features which characterize this virus referred to as the B Virus. In rabbits, depending upon the concentration of the virus (rabbit brain and cord suspension), intracutaneous injection anywhere on the thorax or abdomen gives rise to a necrotic skin-lesion within 1 to 12 days, invariably followed by flaccid paralysis of the trunk and posterior extremities 6 to 17 days after inoculation; all paralysed rabbits die within 1 to 3 days; the adrenals, the spleen and the liver are all frequently involved. The virus gives rise to acidophilic intranuclear inclusion bodies in cells of ectodermal, entodermal and mesodermal origin, of the type seen in infections with pseudorabies (Hurst, 1933), herpes, Virus III, and many other viruses. It is readily filterable through Berkfeld "V" and "N" and Chamberland "L<sub>3</sub>" filters. It is pathogenic for *Macacus rhesus* monkeys, while guinea-pigs and mice are only slightly and irregularly susceptible. A consideration of all its properties indicates that in many respects it is unlike any hitherto described virus. In

its neurotropism, and its capacity to attack tissues derived from all embryonic germinal layers, as well as in the character of its inclusion bodies, it simulates most closely the viruses of herpes simplex and pseudorabies (mad itch, infectious bulbar paralysis). The immunological relationship of the B Virus to these two viruses has therefore been investigated most extensively; the relationship to Virus III (particularly since the B Virus was first established in rabbits and because of the similarity of the inclusions) and to vaccinia was also studied.

#### THE IMMUNOLOGICAL RELATIONSHIP OF THE B VIRUS TO HERPES SIMPLEX.

##### *The Reaction of Herpes-Immune Rabbits to Infection with the B Virus.*

The herpes virus used to infect the rabbits was the highly neurotropic, Perdrau EL<sub>1</sub> strain supplied through the courtesy of Dr. C. H. Andrewes, of the National Institute for Medical Research. Rabbits' testes, removed 48 to 72 hours after inoculation with herpetic brain, gave good skin-lesions upon intracutaneous injection. As a rule, 1 c.c. of a 10 p.c. suspension of herpes testis was injected intracutaneously in 5 zones at weekly intervals, usually for 3 weeks. Approximately 25 p.c. of these rabbits developed encephalitis and died. The surviving ones received about 100 minimal infective doses of herpes intracerebrally two weeks after the last intracutaneous injection, but only 2 out of 6 rabbits were immune by this test. A number of other rabbits, which became immune to intracutaneous inoculation of herpes, were not submitted to the intracerebral test. Another rabbit which recovered from encephalitis following a herpetic kerato-conjunctivitis was also tested for immunity to B Virus. All herpes-immune rabbits were bled about two weeks after the last inoculation of virus. The sera were titrated for their capacity to neutralize herpes virus in the following way: A 10 p.c. suspension in saline of a freshly removed rabbit's testis, inoculated 48 to 72 hours before with herpetic brain, was lightly centrifuged, and various dilutions of the supernatant liquid were mixed with equal quantities of serum, and after about 4 hours' incubation at room temperature 0.2 c.c. was injected on a rabbit's back. Upon each rabbit used for a neutralization test a control titration of the virus was performed; only when the control virus lesions were distinct with definite vesicles were the other results given any consideration. Since, as other investigators have observed, rabbit skins differ somewhat in their reactivity to herpes, many tests had to be repeated.

The herpes-immune rabbits were tested for their reactivity to B Virus at intervals of two weeks to one month after the last inoculation of herpes virus. As far as possible, a skin site was selected where no previous inoculations had been made. Care had to be taken in the selection of nervous tissue for the preparation of the B Virus suspensions, since it was found that at certain stages of the disease (following intracutaneous inoculation) the virus is limited to the spinal cord; in later tests the rabbit was allowed to die, and pieces of cortex, mid-brain, medulla and spinal cord were used for making each suspension. Although by this method practically constant concentrations of virus could be obtained, each test was nevertheless controlled by the simultaneous inoculation of two normal rabbits with what were regarded as limiting doses,

TABLE I.—*The Reaction of Herpes-Immune Rabbits to Infection with the B Virus by the Intracutaneous Route.*

Rabbit No.	History and remarks.	Number of M.I.S.D. of herpes neutralized by 1 c.c. of serum.	Number of B Virus injected.	Result.
99	Had herpetic orchitis, kerato-conjunctivitis and encephalitis; recovered	1000	10, <100	S <sub>12</sub> , Par. 17, D. 19.*
73	Recovered from multiple herpetic skin-lesions. Survived 100 m.i.d. of herpes intracerebrally	1000	100, <1000	S <sub>12</sub> , Par. 8, D. 8.
27	Three series of herpes intracutaneously. Survived 100 m.i.d. of herpes intracerebrally	100, 500 ?	1, <5	S <sub>22</sub> , no Par.; survived.
105	Recovered from multiple herpetic skin-lesions. Immune to second intracutaneous injection of herpes at time of test.	1000	100, <1000	S <sub>22</sub> , Par. 12, D. 16.
111	Two series of herpes intracutaneously	1000	1, <10	S <sub>22</sub> , Par. 12, D. 13.
28	Three series of herpes intracutaneously. Immune to intracutaneous herpes	100, 500	100, <1000	S <sub>22</sub> , Par. 10, D. 11.
29	Ditto	100, 500	1, <5	S <sub>22</sub> , Par. 10, D. 11.
63	"	Not tested	1	S <sub>22</sub> , Par. 6, D. 7.
64	"	"	10, <100	No lesion; survived.
36	"	"	100, <1000	S <sub>22</sub> , Par. 11, D. 13.
5	Four series of herpes intracutaneously. Immune to intracutaneous herpes	?	1, <10	No lesion; survived.
		500	100, <1000	S <sub>22</sub> , Par. 10, D. 12.
			1 ?	S <sub>22</sub> , Par. 11, D. 13.
			10, <100	No lesion; survived.
			100, <1000	S <sub>22</sub> , no Par.; survived.
				S <sub>22</sub> , Par. 8, D. 10.

M.I.S.D. of herpes: Minimal infective skin dose of testicular passage of Perdrau EL<sub>1</sub> strain—minimal amount required to produce a herpetic skin-lesion.

M.I.S.D. of B Virus: Minimal amount of B Virus (brain and cord) required to produce paralysis and death, by the intracutaneous route.

S<sub>12</sub>, Par. 17, D. 19: Skin-lesion 12th day, paralysis 17th day, and death 19th day after injection.

in order to gauge approximately the quantity of B Virus injected into the herpes-immune rabbits. 10 p.c. suspensions were centrifuged at about 2000 r.p.m. for 15 minutes and dilutions were prepared in saline; 0.2 c.c. of the various dilutions were injected intracutaneously. The results are presented in Table I. For a proper understanding of the data it must be stated that the B Virus is unusually virulent for the nervous system of rabbits, in that the lowest effective amount injected intracutaneously produces paralysis and death although not necessarily a grossly discernible skin-lesion; it should be stated also that in control rabbits even the smallest skin-lesion is always followed by paralysis, so that the occurrence of such a lesion without paralysis represents a definite deviation from the normal course of the disease. The results in Table I show that all the herpes-immune rabbits succumbed when tested with more than 10 minimal infective skin doses (m.i.s.d.) of B Virus; 3 rabbits failed to resist 1, < 10 m.i.s.d.,\* and 2 others 10, < 100 m.i.s.d. Four rabbits resisted small doses of B virus; Rabbits 64 and 63 resisted 1, < 10 and 10, < 100 m.i.s.d. respectively; when re-tested 3 weeks later, however, with 100, < 1000 m.i.s.d. they succumbed in the usual manner; Rabbit 27, injected with 1, < 5 m.i.s.d., developed a skin-lesion which faded, but 3 weeks later failed to resist 100, < 1000 m.i.s.d.; Rabbit 5 similarly developed only a skin-lesion with 10, < 100 m.i.s.d., but was not immune to 100, < 1000 m.i.s.d. 3 weeks later. All these rabbits, however, were immune to many thousands of minimal infective doses of herpes given by the intracutaneous route, and the serum of all those tested showed a moderately high content of herpeticidal antibodies. That the B Virus and herpes simplex are not immunologically identical seems to be apparent from these results. The resistance of an occasional herpes-immune rabbit to small doses of B Virus suggests, however, a possible partial relationship between these two viruses; although one can expect that not all animals injected with low concentrations of B Virus may receive the minimally effective amount, experience in controls with the doses used for the herpes-immune rabbits indicates that this possibility is not a likely explanation for the resistance encountered.

*Reaction of a "Herpes-treated" Macacus rhesus Monkey to Infection with B Virus.*

The susceptibility of *Macacus rhesus* to infection with B Virus (to be described in a separate communication), in contradistinction to the resistance of this species to infection with any strain of herpes virus (Zinsser, 1929; McKinley and Douglass, 1930; Cowdry and Kitchen, 1930), constitutes a marked difference between the two viruses. The purpose of this experiment was to determine whether or not preliminary injections of herpes would influence the reaction of a *rhesus* monkey to subsequent infection with B Virus. A normal monkey was bled, and its serum failed to neutralize a single minimal infective skin dose of either herpes or B Virus. It was then injected intracerebrally (1.5 c.c.), intraperitoneally (10.0 c.c.) and intracutaneously (0.6 c.c.)

\* The expression 1, < 10 m.i.s.d. refers to an amount of virus which was certainly one minimum infective skin dose and certainly less than 10 m.i.s.d., as determined by simultaneous control titrations; the actual value may perhaps be 1 or in the range of 2, 3 or 4 m.i.s.d., but not 5 to 10 m.i.s.d.

with uncentrifuged 10 p.c. suspensions of the Perdrau EL<sub>1</sub> and the H.F. strains of herpes. The monkey remained entirely well; there was no fever and no skin-lesions developed (0.2 c.c. of a thick suspension of herpes rabbit testis which produced marked lesions in rabbits was also injected at the same time and failed to produce any lesion in the monkey). Fourteen days later it was bled again and its serum now neutralized herpes virus moderately well (1 c.c. of serum neutralized 100–200 m.i.s.d. of herpes), but not even 2 m.i.s.d. of B Virus. It was then inoculated with a monkey strain of B Virus (monkey brain and cord suspension) intracerebrally, intraperitoneally and intracutaneously; skin-lesions developed, signs of encephalitis appeared on the third day, and the monkey died on the fourth day. Comparison with other monkeys similarly inoculated with B Virus shows that the preliminary injections of herpes and development of herpeticidal antibodies did not modify the course of the disease. This fact is particularly significant, since the preliminary intraperitoneal injection of B Virus renders a monkey immune within 14 days to the otherwise constantly fatal, combined intracerebral and intraperitoneal inoculation. This experiment further indicates that B Virus and herpes simplex are essentially different viruses.

*Reaction of B Virus-Immune Animals to Infection with Herpes.*

In previous tests it was shown that animals immunized with herpes are not generally immune to B Virus. For a more complete understanding of the relationship between the two viruses it was necessary to determine the reverse, *i. e.* the reaction of B Virus-immune animals to infection with herpes. There was, at first, considerable difficulty in this attempt, since all rabbits paralysed with B Virus die, and doses of virus insufficient to produce paralysis do not give rise to immunity. Immunization of rabbits with formalinized B Virus was then attempted; 0.1, 0.15 and 0.2 p.c. formalin (40 p.c. formaldehyde) failed to kill the virus, even after two weeks in the cold; 0.3 and 0.4 p.c. formalin killed the virus, but the rabbit receiving three series of the 0.3 p.c. formalinized virus was not immune to 10, <100 m.i.s.d., while the one receiving 0.4 p.c. formalinized virus resisted 10, <100 but not 100, <1000 m.i.s.d. of B Virus injected intracutaneously.

As was stated previously, guinea-pigs are irregularly and only slightly susceptible to infection with B Virus. It was observed, however, that the apparently resistant guinea-pigs had a varying fever for several days, and that their serum was subsequently capable of neutralizing the virus. Four guinea-pigs, 2 of which were inoculated twice (2 weeks apart) with B Virus, were then tested with a dermatropic guinea-pig strain of herpes (kindly supplied by Dr. M. H. Salaman, of Dr. Bedson's laboratory), intracutaneously on one flank, and scarified on the skin of the other flank. Typical reactions, in no way different from those in a control, developed at all sites in the 4 B Virus-immune guinea-pigs. It is interesting to note, however, that 3 guinea-pigs convalescent from B Virus survived when injected intracerebrally with the Perdrau EL<sub>1</sub> rabbit strain, which is much less virulent for guinea-pigs; 14 days later, 2 of these were tested with Bedson's guinea-pig strain of herpes by scarification and pad inoculation, and developed typical marked reactions.

*Cross-Neutralization Tests with Anti-Herpes and Anti-B Virus Sera.*

The active cross-immunity tests just described indicated that herpes simplex and B Virus are immunologically quite distinct, although the quantitative tests on herpes-immune rabbits pointed to a possible partial relationship. To investigate this relationship further, quantitative neutralization tests were carried out with the immune sera against both viruses. With one exception the herpes antisera were derived from the rabbits which were used for the active immunity tests; the serum designated as "W.S." was a pooled anti-herpes serum derived from many rabbits immunized over a period of months with the Perdrau EL<sub>1</sub> strain and was kindly supplied by Dr. Wilson Smith. The only anti-B Virus sera available at first were from *rhesus* monkeys injected intracutaneously and intraperitoneally with the virus. These sera may also be regarded as convalescent, since marked necrotic skin-lesions resulted at the sites of intracutaneous injection. Evidence for the specific nature of these lesions will be given elsewhere, but it may be stated that re-inoculation after an interval of 7 to 14 days or more resulted in either completely negative or very negligible reactions. The usual procedure was to give 3 injections at weekly intervals, consisting of 1 c.c. intracutaneously in five zones and 10 c.c. intraperitoneally of a 10 p.c. rabbit brain and cord virus suspension. The monkeys were bled 2 weeks after the last inoculation and also subsequently. A great drawback in the use of the convalescent and hyperimmune monkey sera for this purpose was that some normal *rhesus* sera contain variable amounts of herpeticidal antibody, although in the present study they were not found to contain any appreciable anti-B Virus antibody. The availability of sera from B Virus-convalescent guinea-pigs was therefore most helpful.

*Intracutaneous tests.*—The technique of the herpes neutralization tests has already been described; it should be stressed again that only when the control titration on the same rabbit was clean cut, with definite vesiculation of the lesions, were the results considered satisfactory; when such was not the case the titrations were repeated. It may be stated that many tests had to be repeated until the procedure of using only fresh herpes testes removed 48 to 72 hours after inoculation with glycerinated herpetic brain was adopted, when practically constant results were obtainable. For the B Virus neutralization tests large numbers of rabbits had to be used because each dose required an individual rabbit. It is impracticable to attempt to titrate more than one dose on the same rabbit, chiefly because the end-point is paralysis and death rather than a skin-lesion, and furthermore, the animal may be dead from one dose before the skin-lesion of another has had time to appear. A 10 p.c. suspension in saline of glycerinated rabbit brain and cord was usually prepared the day before the test and allowed to stand in the cold room overnight; it was then centrifuged at about 2000 r.p.m. for 10 to 15 minutes. The supernatant liquid was considered as representing a virus concentration of 1:10, and dilutions were made from it accordingly. 0.15 c.c. of the diluted virus was mixed with 0.15 c.c. of serum, and the mixtures were allowed to stand at room temperature for about 4 hours before inoculation. 0.2 c.c. of the mixture was injected intracutaneously on a rabbit's back from which the hair had been clipped. Simultaneously with each test two or more control rabbits were

TABLE II.—Comparative Neutralization Tests with Anti-herpes and Anti-B Virus Sera against Herpes and B Virus Respectively.

Serum.	Source of serum.	Against B Virus.				Against herpes (Perdrau, EL <sub>1</sub> ).			
		Dilution of serum.	Dilution of virus.	Minimal infective skin doses.	Result.	Dilution of serum.	Dilution of virus.	Minimal infective skin doses.	Result.
	Rabbit 27, hyperimmunized	1:2	1:1000	1, <10	S <sub>8</sub> , Par. 11, D. 12*	1:2	1:20	50, <500	Doubtful lesion.
		1:2	1:1000	1, <10	S <sub>8</sub> , Par. 11, D. 12*	1:2	1:100	10, <100	No lesion.
	Rabbit 73, hyperimmunized	1:2	1:20	50, <500	S <sub>8</sub> , Par. 8, D. 10	1:2	1:20	100, <500	"
		1:2	1:100	10, <100	S <sub>8</sub> , Par. 11, D. 13	1:2	1:100	20, <100	"
	"W.S." pooled hyper-immune rabbit serum	1:2	1:20	100, <500	S <sub>8</sub> , Par. 9, D. 11	1:2	1:20	50, <500	"
		1:2	1:200	5, <10	No lesion: survived	1:2	1:100	10, <100	"
	Rabbit 29	1:2	1:20	50, <500	S <sub>8</sub> (sl.), Par. 11, D. 13	1:20	1:20	50, <500	"
		1:2	1:100	10, <100	S <sub>8</sub> , Par. 12, D. 14.	1:30	1:20	50, <250	"
	Rabbit 5	1:2	1:100	1, <10	S <sub>8</sub> , Par. 9, D. 11	1:100	1:100	10, <50	"
		1:2	1:100	1, <10	S <sub>8</sub> , Par. 9, D. 11	1:200	1:20	50, <500	"
	Rabbit 28	1:2	1:100	1, <10	No lesion: survived	1:2	1:20	50, <500	Herpetic lesion.
		1:2	1:100	1, <10	S <sub>8</sub> , Par. 9, D. 11	1:2	1:100	10, <100	Slight herpetic lesion.
	M. rhesus 9, normal	1:2	1:1000	1, <10	D. 8—cause?	1:2	1:100	10, <100	No lesion.
		1:2	1:100	1, <10	No lesion: survived	1:2	1:20	50, <500	"
	M. rhesus 9, 2 weeks after injection of large quantity of herpes virus by intracerebral, intracutaneous and intraperitoneal routes	1:2	1:20	50, <500	S <sub>8</sub> , Par. 9, D. 11	1:2	1:1000	1, <10	"
		1:2	1:100	1, <10	S <sub>8</sub> , Par. 11, D. 12	1:2	1:20	50, <500	Slight herpetic lesion.
		1:2	1:100	1, <10	S <sub>8</sub> , Par. 11, D. 12	1:2	1:20	50, <500	No lesion.
		1:2	1:100	1, <10	S <sub>8</sub> , Par. 11, D. 12	1:2	1:100	10, <100	"
		1:2	1:500	2, <20	S <sub>8</sub> , Par. 8, D. 8	1:2	1:20	50, <500	Herpetic lesion.
		1:2	1:500	2, <20	S <sub>8</sub> , Par. 8, D. 8	1:2	1:100	20, <100	"
		1:2	1:500	2, <20	S <sub>8</sub> , Par. 8, D. 9	1:2	1:1000	2, <10	"
		1:2	1:500	2, <20	S <sub>8</sub> , Par. 8, D. 9	1:2	1:20	100, <500	"
		1:2	1:500	2, <20	S <sub>8</sub> , Par. 8, D. 9	1:2	1:100	20, <100	No lesion.
		1:2	1:500	2, <20	S <sub>8</sub> , Par. 8, D. 9	1:2	1:1000	2, <10	"

Anti-herpes.

<i>M. rhesus</i> 2 before immunizing with B Virus	1:2	1:20	?	S <sub>3</sub> , Par. 7, D. 8	1:2	1:20	5, <50	No lesion.
	1:2	1:200	?	S <sub>4</sub> , Par. 8, D. 9	.	1:100	1, <10	"
<i>M. rhesus</i> 2 after immunizing with B Virus :	1:2	1:1000	5, <50 ?	S <sub>7</sub> , Par. 11, D. 13	.			
† 19.iii.34	1:2	1:100	50, <100	No lesion; survived	1:2	1:20	50, <500	Herpetic lesion.
	1:2	1:20	250, <500	S <sub>8</sub> , Par. 13, D. 14	.	1:100	10, <100	No lesion.
27.iii.34	1:2	1:20	50, <500	S <sub>10</sub> , Par. 11, D. 15	.	1:1000	1, <10	"
	1:10	1:20	50, <500	S <sub>7</sub> , Par. 11, D. 14	.			
	1:20	1:20	50, <500	S <sub>8</sub> , Par. 10, D. 11	.			
<i>M. rhesus</i> 3 before immunizing with B Virus	1:2	1:200	?	S <sub>3</sub> , Par. 7, D. 8	1:2	1:20	50, <500	Herpetic lesion.
	1:2	1:1000	5, <50 ?	S <sub>8</sub> , Par. 9, D. 10	.	1:100	10, <100	"
					.	1:1000	1, <10	"
<i>M. rhesus</i> 3 after immunizing with B Virus :	1:2	1:200	25, <50	No lesion; survived	1:2	1:20	50, <500	"
27.iii.34	1:2	1:20	250, <500	"	.	1:100	10, <100	No lesion.
	1:10	1:20	25, <50	"	.	1:1000	1, <10	"
	1:10	1:20	50, <500	S <sub>7</sub> , Par. 15, D. 16	.			
	1:50	1:30	35, <350	No lesion; survived	1:2	1:20	50, <500	"
	1:10	1:20	25, <50	S <sub>7</sub> , Par. 17, D. 19	.	1:100	10, <100	"
19.iv.34	1:10	1:20	50, <500	No lesion; survived	1:10	1:20	50, <500	"
	1:50	1:20	50, <500	S <sub>2</sub> , Par. 9, D. 11	.			
	1:100	1:20	50, <500		.			
Normal guinea-pig serum	1:2	1:500	2, <20	S <sub>3</sub> , Par. 8, sacrificed	1:2	1:20	100, <500	Herpetic lesion.
					.	1:100	20, <100	"
					.	1:1000	2, <10	"
After immunizing with B Virus	Pooled	1:20	50, <500	S <sub>8</sub> , no Par.; survived	1:2	1:20	100, <500	"
					.	1:100	20, <100	"
					.	1:1000	2, <10	"
Guinea-pig 1†	1:2	1:100	10, <100	No lesion; survived	1:2	1:20	100, <500	"
Guinea-pig 2	1:2	1:500	2, <20	"	.	1:1000	20, <100	"
Guinea-pig 3	1:10	1:20	50, <500	"	.	1:100	100, <500	"
					.	1:1000	20, <100	"
Guinea-pig 4	1:2	1:20	50, <500	No lesion; survived	1:2	1:20	50, <500	"
	1:2	1:100	10, <100	"	.	1:100	10, <100	"
	1:2	1:500	2, <20	"	.	1:1000	1, <10	No lesion.

\* S<sub>6</sub>, Par. 11, D. 12: Skin-lesion 6th day, paralysis 11th day, dead 12th day after inoculation.

† The dates refer to different bleedings from the same monkey.

‡ The sera of guinea-pigs 1, 2 and 3 were tested individually against herpes, but were pooled for the B Virus test.

usually injected—one with 0.2 c.c. of a 1 : 1000 dilution, and another with 0.2 c.c. of a 1 : 10,000 dilution of the virus.

The results are compiled in Table II, in which the titrations of a number of antiherpes and anti-B Virus sera against both viruses are recorded. Of the 7 antiherpes sera tested, not a single one neutralized 10, < 100 m.i.s.d. of B Virus. Of 5 sera tested in the range of 1, < 10 m.i.s.d. of B Virus, 3 showed no effect, while 2 completely neutralized what would appear to be a minimal dose of the virus. The very potent "W.S." serum, which neutralized the highest concentration of herpes, even in a dilution of 1 : 100, neutralized what might have been 5–10 m.i.s.d. of B Virus, but definitely failed to neutralize 10, < 100 m.i.s.d. when used undiluted; it clearly modified the disease produced by the higher concentrations of virus, however, as was evidenced by a delay in the appearance of the skin-lesion and onset of paralysis, or even by the complete inhibition of the skin-lesion, though not of paralysis and death. All the antiherpes sera recorded in the table were prepared with the Perdrau EL<sub>1</sub> strain; it was therefore interesting to determine whether sera against other strains of herpes, particularly dermatotropic ones, would exhibit the same properties with regard to the B Virus. A strongly hyperimmune guinea-pig serum prepared with Bedson's dermatropic guinea-pig strain was obtained through the courtesy of Dr. M. H. Salaman. This serum neutralized herpes, even in a dilution of 1 : 300, and when used undiluted it also completely neutralized 5, < 50 m.i.s.d. of B Virus. The numerous tests which were performed left little doubt that these observations could not be explained on the basis of varying rabbit susceptibility, but suggested rather that strong hyperimmune herpes sera had a partial action against the B Virus.

The results with the anti-B Virus sera clearly show that this virus is readily and completely neutralizable in the rabbit by the intracutaneous route. The sera of two monkeys were titrated against the two viruses, both before and after immunization with B Virus. As has been stated, normal *rhesus* monkeys not infrequently have herpeticidal antibodies in their blood; in comparative tests on 12 monkey sera not recorded in the table, 3 were found to contain herpeticidal antibodies (one in high concentration), but not a single one neutralized 10, < 100 m.i.s.d. of B Virus, while 11 had no effect against even 2, < 10 m.i.s.d. The appearance of neutralizing properties against B Virus in the sera of immunized monkeys is therefore quite significant as a specific response. The serum of monkey 2, which neutralized herpes before infection with B Virus, showed no appreciable rise in its anti-herpes titre during the development of antibodies for B Virus. The serum of Monkey 3 had no antibodies against either virus before, but after immunization showed a rather high titre against B Virus; it is interesting to note, however, that a certain amount of herpeticidal antibody also developed, although the serum (particularly of the first bleeding) was much more effective against B Virus than against herpes. As is shown in the table, further immunization of this monkey increased the titre against herpes. But the origin of herpeticidal antibodies in normal *rhesus* monkeys is not clear; they are not susceptible to infection with herpes, and mere contact with minutes quantities of this ubiquitous virus is not a likely explanation of their presence. Great caution must be exercised, therefore, in correlating their apparent development or

increase in titre with any simultaneous stimulation of the antibody-producing mechanism; thus, Weyer (1932) observed an apparent production of herpeticidal antibodies following experimental poliomyelitis in *Macacus rhesus* monkeys.

It was fortunate, therefore, that an anti-B Virus serum could be prepared in another species. Thus, it was found that 3 convalescent guinea-pig sera which neutralized the highest concentration of B Virus employed failed to neutralize even a single m.i.s.d. of herpes. Another potent anti-B Virus guinea-pig serum (No. 4), however, neutralized what appeared to be a minimal quantity of herpes. It is thus possible to prepare sera which neutralize either

TABLE III.—*Effect of Anti-herpes and Anti-B Virus Sera on Herpes Virus by the Intracerebral Route.*

Serum.	Source of serum.	Dilution of serum.	Dilution of virus.	Result.
None	Control	..	1 : 1000	Signs of encephalitis 6th day; dead 7th.
		..	1 : 10,000	No signs; survived.
		..	1 : 100,000	„ „
Anti-herpes	“ W.S.”	1 : 2	1 : 1000	No signs; survived.
		1 : 2	1 : 100	„ „
		1 : 2	1 : 20	Encephalitis 4th day; dead 5th.
		1 : 10	1 : 20	Signs of encephalitis 4th day; dead 5th.
		1 : 100	1 : 20	No signs; survived.
	Rabbit 27	1 : 2	1 : 200	„ „ *
Anti-B Virus	<i>Macacus rhesus</i> 3, before immunization with B virus	1 : 2	1 : 1000	Signs of encephalitis 4th day; dead 5th.
	<i>Macacus rhesus</i> 3, after immunization with B Virus	1 : 2	1 : 1000	Signs of encephalitis 6th day; dead 6th.
		1 : 2	1 : 100	Signs of encephalitis 4th day; dead 5th.
		1 : 2	1 : 20	Signs of encephalitis 4th day; dead 5th.

\* This apparently paradoxical survival is difficult to explain; none of the material injected had escaped. The rabbit was re-inoculated intracerebrally 3 weeks later with 0.2 c.c. of a 1 : 50 dilution of herpes, developed signs of encephalitis on the 4th day and died on the 5th.

herpes or B Virus exclusively, but it would appear that a very potent anti-herpes serum may neutralize a small amount of B Virus and some potent anti-B Virus sera a small amount of herpes—a phenomenon analogous to that which obtains among anti-bacterial sera for micro-organisms which are not identical, yet have an antigen in common. The results of the active immunity tests taken together with those of the neutralization tests strongly suggest that a similar relationship may well exist between the B Virus and herpes.

*Intracerebral neutralization tests.*—For the herpes intracerebral tests a centrifuged brain suspension was used, and the serum-virus mixtures were incubated for 4 hours at room temperature before inoculation (0.2 c.c.). Table III records the titration of two anti-herpes sera and a monkey anti-B Virus serum against herpes. It may be seen that, although the results are somewhat

irregular and not as good as those of the intracutaneous tests, herpes virus can be neutralized by the intracerebral route when mixed with its own immune serum. The anti-B Virus serum, however, failed to neutralize even a single dose of herpes by this route.

Preliminary tests indicated that B Virus was not neutralized by the intracerebral route when the serum-virus mixtures were incubated for 4 hours at room temperature; the results recorded in Table IV are with serum-virus

TABLE IV.—*Effect of Anti-B Virus and Anti-herpes Sera on B Virus by the Intracerebral Route (Rabbits).*

Serum.	Source of serum.	Dilution of serum.	Dilution of virus.	Result.
None	Control	.	1 : 1000	Signs of encephalitis 4th day; dead 4th.
			1 : 10,000	No signs; survived.
			1 : 100,000	" "
Anti-B Virus	<i>Macacus rhesus</i> 3, after immunization with B. Virus	1 : 2	1 : 1000	Signs of encephalitis 5th day; dead 5th.
		1 : 2	1 : 100	Signs of encephalitis 4th day; dead 4th.
		1 : 2	1 : 20	Signs of encephalitis 4th day; dead 4th.
	<i>Macacus rhesus</i> 2, after immunization with B. Virus	1 : 2	1 : 100	Signs of encephalitis 5th day; dead 5th.
	<i>Macacus rhesus</i> 5, after immunization with B. Virus	1 : 2	1 : 100	Signs of encephalitis 5th day; dead 6th.
		1 : 2	1 : 100	Signs of encephalitis 4th day; dead 5th.
Anti-herpes	" W.S."	1 : 2	1 : 1000	Signs of encephalitis 5th day; dead 6th.
		1 : 2	1 : 100	Signs of encephalitis 4th day; dead 5th.
	Rabbit 5	1 : 2	1 : 1000	Signs of encephalitis 5th day; dead 6th.

mixtures which were incubated for 2 hours at 37° C. and for 22 hours more at 1° C. before inoculation. All the anti-B Virus sera tested definitely neutralized the virus by the intracutaneous route, yet completely failed to show any effect even against a minimal dose of the virus by the intracerebral route in spite of the preliminary 24 hours' incubation. This observation is particularly interesting in view of the fact that the minimal infective dose of B Virus is approximately the same by both the intracerebral and intracutaneous routes, and strongly suggests that the actual inactivation of the virus does not occur *in vitro*. Furthermore, the inactivation of the virus *in vivo* depends upon the tissue into which it is introduced, and is probably influenced by the rapidity with which the susceptible cells are invaded, and the local availability of the other factors necessary for the complete inactivation of the virus. Similar observations have been made with vaccinia (Andrewes, 1928; Fairbrother, 1932, 1933) and Virus III (Andrewes, 1928). Thus, it is quite clear that the demonstration of partial relationships between viruses by means of neutralization tests is probably influenced by the "avidity" of the virus for the

susceptible cells, and that such relationship could not be shown for the B Virus and herpes if the intracerebral route were used for the comparative studies.

#### RELATIONSHIP OF B VIRUS TO PSEUDORABIES.

Clinically and pathologically the B Virus has a great deal in common with that of pseudorabies. The intracutaneous injection in rabbits of either virus in the form of rabbit-brain suspension is followed by necrotic skin-lesions which are practically indistinguishable, grossly as well as microscopically, and the same type of acidophilic intranuclear inclusion body is found in the epithelial cells. Both viruses then invariably reach the spinal cord (that of pseudorabies much more rapidly), and kill the animal by an ascending infection; furthermore, the cardinal symptom of pseudorabies, which is itching in the region corresponding to the level of the spinal cord invaded (Hurst, 1933, 1934), leading to biting of the zone, has also been observed in approximately 25 p.c. of the rabbits injected with B Virus. Both viruses frequently invade the liver, spleen and adrenals, giving rise to histologically indistinguishable lesions. In contradistinction to herpes, both viruses are pathogenic for *Macacus rhesus*; the rabbit is the most susceptible host for both, while pseudorabies is considerably more virulent for guinea-pigs and mice. Filtration experiments with pseudorabies, Iowa strain (Shope, 1931), coincide well with the results on filtration of the B Virus. Still, it must be stressed that, if only because of the greater virulence and the constant occurrence of "itching" with pseudorabies, there is no difficulty in distinguishing it clinically from the B. Virus. It was therefore most important to determine the immunological relationship between these two viruses.

The pseudorabies virus used in this work was the virulent Aujeszky strain. Passive cross-immunity tests were performed with the various anti-B Virus sera which were available, and one anti-pseudorabies swine serum which was obtained through the courtesy of Dr. R. E. Shope, of the Rockefeller Institute, Princeton, N.J. The only possible active immunity tests were with B Virus-immune animals, and for those one *rhesus* monkey and a number of guinea-pigs were employed.

#### *Cross-Neutralization Tests with Anti-pseudorabies and Anti-B Virus Sera.*

*Effect of anti-B Virus sera on pseudorabies in guinea-pigs.*—Shope (1931, 1932) pointed out that pseudorabies is so virulent for rabbits that neutralization is not readily demonstrable in them, even when the serum-virus mixtures are injected subcutaneously, and that the best results are obtained when the virus in the form of rabbit brain is mixed with the serum and injected subcutaneously in guinea-pigs. A 10 p.c. rabbit pseudorabies brain suspension was allowed to sediment for several hours or centrifuged lightly, and the opalescent supernatant liquid was taken as virus 1 : 10 and dilutions made from it accordingly. To 1 c.c. of the various dilutions of virus the indicated amounts of serum were added, and, after incubation at room temperature for 2 hours and at 1° C. overnight, the mixtures were injected subcutaneously into guinea-pigs. Each test was controlled by the inoculation of guinea-pigs with virus

only. Three anti-B Virus monkey sera, 3 normal monkey sera, 1 pooled anti-B virus guinea-pig serum and a swine anti-pseudorabies serum were tested. The results are presented in Table V; the titrations of the virus performed simultaneously are recorded in Table VII. Only about half the control guinea-pigs injected with 1 c.c. of a 1 : 1000 dilution of the virus succumbed, whereas

TABLE V.—*Effect of Anti-B Virus Sera on Pseudorabies in Guinea-pigs.*

Serum.	Amount c.c.	Dilution of pseudorabies.	Amount c.c.	Guinea- pig No.	Result.	Date of test.
None ; saline	1.0	1 : 10	1.0	11	D. 70	29.v.34.†
	1.0	1 : 100	1.0	12	D. 70	
	1.0	1 : 1000	1.0	13	D.< 92*	
<i>Mac. rhesus</i> 3, before immunization with B Virus	1.0	1 : 100	1.0	14	D. 70	
<i>Mac. rhesus</i> 3, 2 months after beginning of immunization with B Virus	1.0	1 : 10	1.0	15	D. 96	
	1.0	1 : 100	1.0	16	Survived	12.vi.34.
	1.0	1 : 1000	1.0	17	"	
<i>Mac. rhesus</i> 3, 3½ months after beginning of immunization with B Virus	1.0	1 : 10	1.0	50	D.< 94	
	1.0	1 : 100	1.0	49	Survived	
	1.0	1 : 1000	1.0	48	"	
<i>Mac. rhesus</i> 2, 6 weeks after beginning of immunization with B Virus	1.0	1 : 10	1.0	53	D. 100	
	1.0	1 : 100	1.0	52	D.< 94	
	1.0	1 : 1000	1.0	51	Survived	
<i>Mac. rhesus</i> 5, 2½ months after beginning of immunization with B Virus	1.0	1 : 10	1.0	56	"	
	1.0	1 : 100	1.0	55	"	
	1.0	1 : 1000	1.0	54	"	20.vi.34.
	0.5	1 : 10	1.0	70	D.< 70	
	1.0	1 : 100	1.0	69	D.< 70	
	1.0	1 : 1000	1.0	68	D.< 70	29.vi.34.
	1.0	1 : 10	1.0	83	D. 72	
	1.0	1 : 100	1.0	82	D. 72	12.vi.34.
<i>Mac. rhesus</i> 9, normal	1.0	1 : 100	1.0	47	Survived	
	1.0	1 : 1000	1.0	46	"	20.vi.34.
	1.0	1 : 10	1.0	65	D. 72	
	1.0	1 : 100	1.0	64	D.< 96	
<i>Mac. rhesus</i> 10, normal	1.0	1 : 100	1.0	67	D.< 72	
	1.0	1 : 1000	1.0	66	D. 120	29.vi.34.
Pooled anti-B Virus guinea-pig serum	0.5	1 : 100	0.5	80	D.< 140	
	0.5	1 : 100	1.0	81	D. 124	20.vi.34.
Swine anti-pseudorabies serum	0.1	1 : 10	1.0	72	Survived	
	0.1	1 : 100	1.0	71	"	

\* D.< 92 found dead 92 hours after inoculation, evidence of pseudorabies infection being signs of biting the inoculated zone.

† See Table VII under date for control titration.

practically all died with 0.5 c.c. of the 1 : 100, and all with 1 c.c. of 1 : 100 dilution or more. For this reason the only changes which were regarded as significant were in guinea-pigs injected with serum-virus mixtures containing more than 1 c.c. of a 1 : 1000 dilution of virus. It may thus be seen that whereas the serum of Monkey 3, before immunization, had no effect whatever,

the serum from two separate bleedings following immunization with B Virus definitely neutralized what may be regarded as a constantly effective minimal quantity of pseudorabies. The serum of Monkey 2, which was relatively much less potent against B Virus, had no effect. The serum of Monkey 5 neutralized pseudorabies on one occasion, but not on two others. With the smaller quantities of guinea-pig serum there was a definite delay in the onset of signs and death, but not complete neutralization. On the basis of these data alone it is quite obvious that the two viruses are not immunologically identical, but the partial effect on minimal quantities of pseudorabies, just as in the case of herpes, suggests a possible relationship.

*Effect of anti-pseudorabies serum on B Virus.*—Only one anti-pseudorabies swine serum was available for this test; normal swine serum was used as a control. The titration was carried out as previously described, equal quantities of the undiluted serum being mixed with various dilutions of virus, as shown in Table VI. Although in the quantities used the anti-pseudorabies serum

TABLE VI.—*Effect of Anti-pseudorabies Serum on B Virus.*

Serum.	Dilution of B Virus.	Rabbit No.	Result.
None—control	1 : 10,000	169	No skin-lesion; survived.
	1 : 1000	158	S <sub>3</sub> (marked), Par. 8, sacrificed.
Normal swine	1 : 1000	189	S <sub>3</sub> (marked), Par. 8, D. 9.*
Anti-pseudorabies swine	1 : 1000	190	S <sub>4</sub> (slight), Par. 10, D. 12.
	1 : 200	191	S <sub>6</sub> , Par. 9, D. 11.
	1 : 100	192	S <sub>7</sub> , Par. 9, sacrificed.

\* S<sub>3</sub>, Par. 8, D. 9: Skin-lesion 3rd day, paralysis 8th day, dead 9th day after inoculation.

failed to neutralize B Virus completely, it seemed to have modified the course of the disease, as was evidenced by the definite delay in the development of the skin-lesion and paralysis when compared with that in control rabbits, as well as with the one receiving the normal swine serum virus mixture.

*Reaction of B Virus-Convalescent Guinea-pigs to Infection with Pseudorabies.*

Eight guinea-pigs which had been inoculated with B Virus by various routes at different times, as shown in Table VII, were injected with varying amounts of pseudorabies subcutaneously. When these are compared with the control guinea-pigs, it becomes quite clear that the majority of them resisted the almost constantly effective minimal dose, 0·5 c.c. of a 1 : 100 dilution, and 2 were immune even to 1 c.c. of a 1 : 100 dilution of pseudorabies. In view of the fact that with the exception of guinea-pig 24 (which, however, also resisted 0·5 c.c. of a 1 : 100 dilution) all the others received their B Virus in the form of rabbit brain and cord, and that the pseudorabies with which they were subsequently tested was also in the form of rabbit brain, it was desirable to determine whether or not any possible development of antibodies against normal rabbit brain might influence the reaction of the animal to the virus. Two guinea-pigs (76 and 77) were injected intraperitoneally with 1 c.c. of a

TABLE VII.—*Reaction of B Virus Convalescent Guinea-pigs to Infection with Pseudorabies.*

Guinea-pig No.	History of B Virus infection.	Date of test.	Amount of pseudorabies subcutaneously	Result.	NORMAL CONTROLS.		
					Date.	Guinea-pig No.	Amount of pseudorabies subcutaneously.
1	23.iv.34: Rabbit virus i.cut. and into left pad. 1.v.34: Flaccid paralysis of post extr. 2.v.34: Chewed away injected foot. 15.v.34: Virus i.cer., i.p., and i.cut.*	5.vi.34 12.vi.34	1.0 c.c., 1:1000 1.0 c.c., 1:100	Survived. D.<94.	5.vi.34	25 26 27	1.0 c.c., 1:10,000 1.0 c.c., 1:1000 1.0 c.c., 1:100
9	Rabbit virus 26.v.34 i.cut., s.cut., and i.cer.	12.vi.34 20.vi.34	0.5 c.c., 1:100 1.0 c.c., 1:100	Survived. D.<96.	12.vi.34	43 44 45	1.0 c.c., 1:10,000 1.0 c.c., 1:1000 1.0 c.c., 1:1000
2	23.iv.34: Same as guinea-pig 1; remained well. 15.v.34: Same as guinea-pig 1. 12.vi: Tested with herpes	20.vi.34	0.5 c.c., 1:100	D. 120.	20.vi.34	61 62 63 66 67	1.0 c.c., 1:1000 1.0 c.c., 1:1000 1.0 c.c., 1:1000 1.0 c.c., 1:100 0.5 c.c., 1:100
3	15.v.34: Rabbit virus i.cut., i.cer., and i.p. 12.vi: Tested with herpes	20.vi.34 29.vi.34 6.vii.34	0.5 c.c., 1:100 1.0 c.c., 1:100 1.0 c.c., 1:10	Survived. " D.<92.	29.vi.34	73 74 75 76 77	1.0 c.c., 1:1000 0.5 c.c., 1:100 0.5 c.c., 1:100 0.5 c.c., 1:100 0.5 c.c., 1:100
4	Same as guinea-pig 3	20.vi.34 29.vi.34 6.vii.34	0.5 c.c., 1:100 1.0 c.c., 1:100 1.0 c.c., 1:10	Survived. " "	29.vi.34	62 63 64 65 66 67	1.0 c.c., 1:1000 1.0 c.c., 1:1000 1.0 c.c., 1:1000 1.0 c.c., 1:100 0.5 c.c., 1:100 0.5 c.c., 1:100
24	2.vi.34: Guinea-pig virus i.cut., i.cer., s.cut.	20.vi.34 29.vi.34	0.5 c.c., 1:100 1.0 c.c., 1:100	" D.<96.	29.vi.34	73 74 75 76 77	1.0 c.c., 1:1000 1.0 c.c., 1:1000 1.0 c.c., 1:100 0.5 c.c., 1:100 0.5 c.c., 1:100
78	12.vi-16.vi.34: 1 c.c. rabbit virus i.p. daily. 25.vi: Flaccid paralysis of post extr.; survived	29.vi.34 6.vii.34	0.5 c.c., 1:100 1.0 c.c., 1:100	Survived. D.<92.	6.vii.34	62 72 73 74	1.0 c.c., 1:1000 1.0 c.c., 1:1000 1.0 c.c., 1:100 1.0 c.c., 1:100
79	12.vi-16.vi.34: 1 c.c. rabbit virus i.p. daily. Remained well.	29.vi.34	0.5 c.c., 1:100	D. 80.		43 74	1.0 c.c., 1:100 1.0 c.c., 1:100

D.<94: Found dead 94 hours after injection with evidence of biting inoculated zone.

\* i.cut., intracutaneously; i.cer., intracerebrally; i.p., intraperitoneally.

† Guinea-pigs 76 and 77 had a series of normal brain injections from 12.vi to 16.vi.34.

10 p.c. suspension of glycerinated normal rabbit brain daily for 5 consecutive days, and 14 days after the last inoculation were tested with 0.5 c.c. of a 1 : 100 dilution of pseudorabies rabbit brain, simultaneously with a number of B Virus convalescent guinea-pigs. The former (76 and 77) succumbed typically, while most of the latter were resistant. It thus appears that in this case, at least, possible tissue antibodies were not responsible for the partial resistance observed.

*Reaction of a B Virus-Immune Rhesus Monkey to Infection with Pseudorabies.*

On 14.iii.34 Monkey 5 was injected intracerebrally and intracutaneously with a monkey strain of B Virus ; it developed marked necrotic skin-lesions and signs of cerebral involvement, but recovered. Fourteen days later, 28.iii.34, it resisted a combined intracerebral and intraperitoneal inoculation of B Virus, which proved fatal for an untreated monkey ; furthermore, a second intracutaneous injection of virus given simultaneously produced no reaction. One month later it was given more B Virus intravenously without any reaction whatever ; it was thus proved solidly immune to B Virus. Its serum neutralized B Virus, and the results of the test against pseudorabies are shown in Table VI. On 31.v.34 it was injected intracerebrally with 1 c.c. of a 10 p.c. suspension of pseudorabies rabbit brain and intracutaneously with 0.4 c.c. of the same suspension. The monkey had no reaction of any kind (daily temperatures revealed no fever), and remained entirely well over a period of weeks. This finding is particularly significant in view of the fact that all the *rhesus* monkeys inoculated by Hurst (1933) with the same dose of pseudorabies invariably succumbed. It should also be noted that any possible tissue immunity played no part here, since the B Virus was given in the form of monkey brain and the pseudorabies in the form of rabbit brain. Pseudorabies is much less virulent for the monkey than for the rabbit, however, since peripheral inoculation is practically without effect ; it is thus quite possible that the dose of pseudorabies administered was within the minimal effective range, and the possession of even a partial immunity was sufficient to render the animal completely resistant. This finding is analogous to the resistance of B Virus-immune guinea-pigs to infection with the rabbit Perdrau EL<sub>1</sub> strain of herpes, but not to the more virulent (for guinea-pigs) Bedson's dermotropic guinea-pig strain. It may, perhaps, be interesting to note that another B Virus-immune monkey failed to resist an inoculation of equine encephalomyelitis given by Dr. Hurst.

The data on the immunologic relationship between the B Virus and pseudorabies are thus quite similar to those on its relationship to herpes, and in both instances it is difficult to escape the conclusion that these viruses, although immunologically specific, are yet somehow related.

IMMUNOLOGIC RELATIONSHIP OF B VIRUS TO VIRUS III.

The strain of Virus III used in these tests was kindly supplied by Dr. C. H. Andrewes, and although it had been in the form of a dry powder for over four years, no difficulty whatever was encountered in demonstrating the presence

of the virus, even in the first testicular passage (typical intranuclear inclusion bodies were present in sections). The subsequent testicular passages yielded suspensions which gave characteristic skin reactions. Three anti-B Virus monkey sera were tested against various dilutions of Virus III by the intracutaneous route; the serum-virus mixtures were incubated for 2 hours at 37° C. before inoculation. The extent of the reactions recorded on the seventh day after inoculation are shown in Table VIII. The anti-B Virus sera failed to neutralize even a minimal dose of Virus III; the complete neutralization by an anti-Virus III serum is evidence for the specificity of the skin-reactions.

TABLE VIII.—*Effect of Anti-B Virus Sera on Virus III.*

Serum.	Concentration of Virus III.			
	1 : 20.	1 : 100.	1 : 500.	1 : 2500.
None—control	++++*	++++	+++	++
Anti-Virus III	—	—	—	—
<i>Mac. rhesus</i> 3, before immunization with B Virus	++++	++++	+++	++
<i>Mac. rhesus</i> 3, after immunization with B Virus	++++	++++	+++	++
<i>Mac. rhesus</i> 2, after immunization with B Virus	++++	++++	+++	++
<i>Mac. rhesus</i> 5, after immunization with B Virus	++++	++++	+++	++

\* The plus marks refer to the extent of the lesions; the reactions with the 1 : 2500 dilution did not appear until the 5th or 6th day after injection.

Two anti-Virus III sera (one from a Brown-Pearce tumour rabbit was supplied by Dr. C. H. Andrewes) failed to neutralize a minimal dose of B Virus. A rabbit, which was tested with a minimal dose of B Virus 15 days after infection with Virus III by the testicular and dermal routes, developed a typical skin-lesion followed by paralysis and death. Thus no immunological relationship could be shown between Virus III and the B Virus.

#### IMMUNOLOGIC RELATIONSHIP OF B VIRUS TO VACCINIA.

Although the tests on vaccinia-convalescent rabbits and with antivaccinal serum were carried out chiefly as controls for the work with herpes and pseudorabies, the negative results are important in the elimination of what might have been assumed (even though remotely) as a possible admixture of vaccinia in the B Virus, particularly in view of the similarity in the clinical pictures produced in the *Macacus rhesus* monkey. Two hyperimmunized and 7 convalescent vaccinia rabbits were tested with various doses of B Virus at intervals varying from 13 to 54 days after the last inoculation of vaccinia. The results recorded in Table IX show that recovery from vaccinia had no influence on the course of B Virus disease in rabbits. Furthermore, a potent antivaccinal serum failed to neutralize even a minimal dose of B Virus. These data further suggest that the results pointing to a partial relationship between the B Virus, herpes and pseudorabies are not readily accounted for on the basis of a probable non-specific immunity.

TABLE IX.—*The Reaction of Vaccinia-Immune Rabbits to Infection with B Virus by the Intracutaneous Route.*

Rabbit No.	History.	Interval between last vaccination and test.	Dilution of B Virus.	Result.
12 .	Four series of vaccinia inoculations	17 days .	1 : 10 .	S <sub>3</sub> , Par. 7, D. 9.*
11 .	Ditto	17 " .	1 : 100 .	S <sub>3</sub> , Par. 8, D. 9.
10 .	Multiple vaccinia skin-lesions	54 " .	1 : 10 .	S <sub>1</sub> , Par. 8, D. 9.
9 .	Ditto	28 " .	1 : 100 .	S, Par. 9, D. 9.
35 .	"	17 " .	1 : 200 .	S <sub>3</sub> , Par. 9, D. 10.
20 .	"	14 " .	1 : 500 .	S <sub>3</sub> , Par. 10, D. 11.
21 .	"	13 " .	1 : 1000 .	S <sub>3</sub> , Par. 12, D. 13.
22 .	"	13 " .	1 : 5000 .	S <sub>2</sub> , Par. 13, D. 13.
43 .	"	15 " .	1 : 5000 .	S <sub>3</sub> , Par. 11, D. 14.

\* S<sub>3</sub>, Par. 7, D. 9 : Skin-lesion 3rd day, paralysis 7th day, dead 9th day after inoculation.

#### ATTEMPTED APPLICATION OF ANTIBODY ABSORPTION TO THE STUDY OF IMMUNOLOGIC RELATIONSHIPS AMONG VIRUSES.

Absorption of antibody has been applied extensively to the study of immunologic relationships among the ordinary bacteria, and has greatly aided in establishing the existence of common antigens among various micro-organisms. It was therefore important to determine whether or not the same methods could be applied to the study of the relationship among the viruses in question. Smith (1930) reported successful specific absorption of antibody with the viruses of vaccinia and herpes ; Gay and Holden (1933), on the other hand, were unable to demonstrate specific absorption. In the present study three attempts were made to absorb the herpeticidal antibody with herpes virus from two anti-herpes rabbit sera, following Smith's technique in practically all details, but without success. Serum-virus mixtures containing a large excess of herpes virus showed no appreciable specific decrease in the antibody titre after removal of the excess virus by filtration. A difficulty encountered in the present tests was the relatively greater filterability of the B Virus. Smith found no herpes virus in the filtrates when he filtered his serum-virus mixtures through Chamberland L<sub>2</sub> candles ; B Virus, however, passed Chamberland L<sub>3</sub> candles. In one test in which equal amounts of a diluted anti-herpes serum were treated with equal amounts by weight of herpes, B Virus and vaccinia testes, the various mixtures were filtered through single disc Seitz filters ; all the filtrates neutralized herpes virus, but on the seventh day, when the control herpes reactions had already begun to fade, skin-lesions appeared at all the sites where the B Virus filtrate was injected ; two days later the rabbit developed paralysis and died. It was thus apparent that while herpes and vaccinia were retained by the filter, B Virus was not, and that a mixture which contained enough herpeticidal antibody to neutralize the highest concentration of herpes, failed to inhibit the B Virus reactions. In this experiment the mixtures were left in contact with frequent shaking at room temperature for 5 days, in another at 37° C. for 3 days, and in the third at 37° C. for 3 days and another 24 hours at 1° C., but

in none was there any evidence of specific, not to say quantitative, antibody absorption. Essentially the nature of an in-vitro reaction between filterable viruses and their antibodies is still obscure. At any rate, the method of antibody absorption was not found to be applicable in the present study.

#### DISCUSSION.

The B Virus recently isolated from a human case of ascending myelitis associated with visceral necrosis, although readily distinguishable from them, has certain features in common with the generally less virulent herpes simplex and the more virulent pseudorabies viruses. All three are neurotropic in the rabbit, and, in addition, capable of attacking, in varying degree, tissues derived from all the embryonal layers; histologically, the lesions produced by the three are practically indistinguishable both in character and in the appearance of the intranuclear inclusion bodies. In the present study the immunological relationship of the B Virus to herpes, pseudorabies, Virus III and vaccinia has been investigated more or less quantitatively. No relationship whatever was found between the B Virus with either Virus III or vaccinia. In the case of B Virus and herpes, however, it was possible to obtain immune sera which neutralized either one virus or the other exclusively; on the other hand, certain sera were also available, which, while possessing a high titre against one, were capable of neutralizing a minimal quantity of the other virus, and *vice versa*. Similarly, most rabbits highly immune to herpes were not resistant to B Virus given by the same route, while a few definitely resisted only minimal quantities of it; in a qualitative test B Virus-immune guinea-pigs failed to resist herpes. In this connection it is necessary to state that Gay and Holden (1933) have also isolated a virus from the same human material (they called it the "W" virus), which, from their description, one may consider as being identical with the B Virus. They concluded that their virus was serologically identical with herpes. Although it is impossible to analyse their results, because the report contains no details, it appears that their conclusions were based on insufficient data, consisting chiefly of comparative neutralization tests with human and monkey sera; they state, however, that their specific antiherpes rabbit sera failed to neutralize their "W" virus, but rather only modified it. Furthermore, one of their "normal" monkey (*Macacus rhesus*) sera neutralized only their "W" virus but not herpes; in view of this fact, and also because they were unable to infect *rhesus* monkeys (while the same species of monkey in England was readily infected with the B Virus), it is not improbable that many of their monkeys might have had natural infections with this virus and thus complicated the comparative serological studies. The quantitative titrations carried out in the present investigation leave little doubt of the essential immunologic difference between the B Virus and herpes, although the data point to a partial relationship.

A similar relationship, however, was discovered between the B Virus and pseudorabies. Guinea-pigs immune to B Virus were shown to resist minimal but constantly infective amounts of pseudorabies. A *rhesus* monkey (a species for which both viruses are much less virulent) hyperimmunized with B Virus

completely resisted an intracerebral inoculation of pseudorabies. Potent anti-pseudorabies serum failed to neutralize B Virus completely, but apparently modified the disease caused by it. A potent anti-B Virus serum, however, was capable of neutralizing a minimal but constantly infective amount of pseudorabies.

In the immunologic relationship of the B Virus to pseudorabies and herpes, there is thus an instance which closely parallels the cases of antigenic relationship among the ordinary bacteria: an instance, however, which has no exact parallel among the so-called "classical" viruses. In the case of the filterable fowl tumours, Andrewes (1931, 1933) observed a distinct immunologic relationship, though not identity, among tumours with entirely different histological structure. Shope (1932*b*) described an immunologic relationship between a filterable virus causing a "tumour-like condition" (fibroma) in rabbits with the virus of infectious myxomatosis. With regard to the relationship among fowl tumours, Andrewes (1931) wrote that "it is, however, pertinent to recollect that if one accepts the unity of the vaccinia-variola virus, there is no instance in the field of acknowledged viruses of a neutralizing serum which can inactivate a heterologous virus". Such an instance, it would appear, is now supplied by the relationship of the B Virus with herpes and pseudorabies. Studies now in progress on the relationship between pseudorabies and herpes suggest that these three viruses may be found to form a species, of which the individuals may perhaps be considered as the "type-specific" members. Among other viruses some are known which are apparently identical in all respects but are distinct immunologically; thus there are two strains of equine encephalomyelitis (Ten Broeck and Merrill, 1933), at least three immunologically distinct types of foot and mouth disease, two of vesicular stomatitis, and according to recent studies by Olitsky, Cox and Syverton (1934), the viruses of equine encephalomyelitis and vesicular stomatitis are practically identical in their properties, but distinct immunologically. It does not appear improbable, however, that quantitative serological investigations of all these viruses may perhaps reveal the existence, at least among some of them, of a certain group relationship.

#### SUMMARY.

1. The immunological relationship of the B Virus to herpes simplex, pseudorabies (mad itch, infectious bulbar paralysis), Virus III and vaccinia has been investigated.

2. Anti-B Virus and antiherpes sera were obtained which neutralized either one virus or the other exclusively, and qualitative tests with moderate doses of virus revealed no active cross-immunity between the two viruses.

3. Quantitative neutralization tests as well as titrations on actively immune animals, however, revealed a partial immunological relationship between B Virus and herpes.

4. A partial immunological relationship was also found to exist between B Virus and pseudorabies.

5. No relationship was found between B Virus and Virus III or vaccinia.

6. The B Virus is therefore regarded as an immunologically distinct and specific filterable virus with a group relationship to herpes and pseudorabies.

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## STUDIES ON THE B VIRUS. II: PROPERTIES OF THE VIRUS AND PATHOGENESIS OF THE EXPERIMENTAL DISEASE IN RABBITS.

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THE human disease from which the B Virus was isolated and some of the properties of the virus were described in an earlier communication (Sabin and Wright, 1934). The immunological investigations on the B Virus (Sabin, 1934) revealed that it is an immunologically distinct entity with a partial relationship to the viruses of herpes simplex and pseudorabies ("mad itch", infectious bulbar paralysis). The purpose of the present communication is to present results of a more detailed study of its properties.

*Preservation in glycerol.*—The B Virus in the form of rabbit brain and cord has been found to retain its activity very well even after prolonged storage in 50 p.c. glycerol. Two specimens were tested after they had been in glycerol for about a year in the ice-chest, and for over two weeks preceding the test at room temperature, and were found to be very potent.

*Method of passage.*—Since its isolation in November, 1932, the B Virus